EXHIBIT 3 (CONT'D)

Table 1
Study GF5503
Primary Endpoint
Matured follicles (at least 14mm)

	Gonal-F		Metrodin		Difference+	95% Confidence Interval++
Center	N	Mean	N	Mean	M - G	
1	3	10.67	4	11.50	.83	(-6.06,7.73)
2	8	5.25	12	5.58	.33	(-1.30,1.97)
3	12	9.25	12	9.67	.42	(-2.41,3.24)
4	3	9.00	1	11.00	2.00	(-11.15,15.15)
5	2	8.00	4	15.00	7.00	(-3.75,17.75)
6	6	6.00	4	7.75	1.75	(-4.18,7.68)
7	12	8.58	12	11.08	2.50	(-1.58,6.58)
8	6	10.17	7	11.29	1.12	(-3.53,5.76)
9	8	5.25	7	5.57	.32	(-2.06,2.71)
Total	60	8.32	63	9.67	1.35	(.09,2.61)*

\+ Metrodin minus Gonal-F. Positive difference favors Merodin over Gonal-F.

++ 95% confidence interval for the difference between Metrodin and Gonal-F (Metrodin minus Gonal-F).

* Confidence interval excludes zero which indicates a statistically significant difference in favor of Metrodin over Gonal-F. p-value=.0365

Treatment-by-Center p-value=.7233

Table 2
Study GF5503
Secondary Endpoints

	Gonal-F		Metrodin		Difference+	95% Confidence Interval++
Endpoint	N	Mean	N	Mean	M - G	
Follices greater than 10mm	60	10.45	63	11.55	1.09	(-.50,2.68)
Number of oocytes recovered	55	9.32	59	10.17	.85	(-.93,2.62)
Number of oocytes fertilized	55	5.44	59	6.05	.61	(-.92,2.14)
Number of cleaved embryos	53	4.59	52	5.60	1.01	(-.26,2.28)

P-values

Endpoint	Treatment	Treatment-by-Center
Follicles greater than 10mm	.1768	.5898
Number of oocytes recovered	.3449	.8058
Number of oocytes fertilized	.4282	.7455
Number of cleaved embryos	.1172	.9180

\+ See Table 1

++ See Table 1

Table 3
Study GF5503
Local Injection Reactions

Itching

	None	Mild	Moderate	Severe	Score+	P-value
Gonal-F	54	1	3	0	.12	.167
Metrodin	55	3	1	0	.08	

Swelling

	None	Mild	Moderate	Severe	Score+	P-value
Gonal-F	52	5	1	0	.12	.201
Metrodin	57	0	2	0	.07	

Redness

	None	Mild	Moderate	Severe	Score+	P-value
Gonal-F	47	9	2	0	.22	.043*
Metrodin	54	4	1	0	.10	

Bruising

	None	Mild	Moderate	Severe	Score+	P-value
Gonal-F	40	13	3	2	.43	.194
Metrodin	46	11	2	0	.25	

Pain

	None	Mild	Moderate	Severe	Score+	P-value
Gonal-F	43	6	3	6	.52	.942
Metrodin	41	11	7	0	.42	

+ Mean score based on None = 0, Mild = 1, Moderate = 2, Severe = 3. Low score desirable.
* p=.043 in favor of Metrodin over Gonal-F.

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: MAR 1 1994

FROM: Mathematical Statistician (HFD-713)

THROUGH: Satya D. Dubey, Ph.D.
Chief, Statistical Evaluation and Research Branch

SUBJECT: Improper Statistical Method for Establishing Therapeutic Equivalence, as Employed in NDA #20-378

TO: File (NDA #20-378, GONAL-F™)

The following concerns and observations were apparent with respect to the above NDA:

1. The sponsor stated in the forwarding letter,.

> "The purity of Gonal-F and complete absence of contaminating LH or other urinary proteins will make available to physicians and patients for the first time a highly purified, monotherapeutic human gonadotropin product. Elimination of the urinary source also enhances safety. As a result of these significant improvements, we propose that GONAL-F replace Metrodin in the marketplace. ... 3. Equivalent efficacy and safety has been demonstrated between GONAL-F and Metrodin in a comparative, adequate and well-controlled clinical study (Study GF 5503)."

Unfortunately, the sponsor applied the usual Tests of Equality, appropriate for establishing a <u>difference</u>, for the purpose of establishing <u>equivalence.</u> The theory behind the usual Test of Equality is such that when a significant difference at 5% level is detected, we can state, in a way, with 95% confidence that the two drugs are not equivalent. However, the theory is such that, when the null hypothesis of equality is not rejected it does not imply with such a confidence that the two drugs are equivalent.

Although the sponsor has indicated that "equivalent efficacy" has been shown, the regulatory requirement for active-controlled trials, in general, is to show that the test drug is not worse than the control product by a clinically meaningful amount (the clinically meaningful difference being specified by FDA clinicians). The appropriate methodology for analyzing an active-controlled study is to calculate 95% confidence intervals for the pertinent efficacy parameters, then to check whether clinically important differences in favor of the control product are ruled out (i.e., are outside the calculated confidence

interval).

2. This reviewer did not see the p-values for comparing treatment groups with respect to Demographic Characteristics. There was a statistically significant difference between Gonal-F and Metrodin groups with respect to Baseline semen analysis of male partners (Progressive motility, p=0.03). This imbalance and any other imbalance found in any Baseline characteristics or possible confounders should be accounted for in the statistical analyses.

3. This reviewer did not see the graphical presentation of 95% confidence intervals for the difference between the two treatment groups with respect to efficacy, side-by-side for overall and all centers separately. This type of graphical presentation helps in assessing the consistency of results across the centers. The following order is preferable: overall, the largest center, the next largest center,..., the smallest center. This is to be done for both "All Patients" and "Evaluable Patients" analyses.

4. A thorough statistical review of this NDA will require data on floppy diskettes (as SAS data sets to expedite the review), i.e., data on baseline and other covariates, and efficacy data, along with the printout of the data and the descriptions of variable names on SAS data sets.

Dipa Choudhury 2-25-94
Japobrata Choudhury, Ph.D.
Mathematical Statistician

Concur: Dr. Nevius 2-25-94

CC:
Original: NDA 20-378 2-25-94
HFD-510
HFD-510/Dr.Sobel
HFD-510/Dr.Corfman
HFD-510/Dr.Bennett
HFD-510/Ms.Braithwaite
HFD-713/Dr.Dubey [File:DRU 1.3.2]
HFD-713/Group 2 File
HFD-713/Dr.Choudhury
Chron.
J.Choudhury:SERB:3-4594:02-03-94

This memorandum consists of two pages.

Human Reproduction vol.15 no.2 pp.2[illegible] 4, 2000

Recombinant follicle stimulating hormone in in-vitro fertilization treatment—clinical experience with follitropin alpha and follitropin beta

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The objective of this prospective study was to compare the outcome of ovarian hyperstimulation for in-vitro fertilization (IVF) using two different preparations of recombinant follicle stimulating hormone (FSH). The study was based on 296 consecutive IVF cycles in 1997, 199 performed using follitropin alpha (Gonal-F) and 97 performed using follitropin beta (Puregon). Outcome was compared regarding pregnancy rate, oestradiol and progesterone response, endometrial thickness, follicle number, number of retrieved oocytes, fertilized oocytes, sperm count and sperm motility. There was no significant difference in outcome of stimulation. Clinical pregnancy rate was similar, 29.1% for Gonal-F and 28.1% for Puregon. There was no difference in endometrial response, oestradiol response, number of smaller (12–15 mm) or larger (>15 mm) follicles, number of oocytes retrieved, fertilized, divided and replaced, in sperm counts or in sperm progressive motility. There was a lower follicle number in the Puregon group, but not statistically significant. The serum progesterone concentrations on the day of oocyte retrieval, however, were significantly lower in the Puregon group. In conclusion, it was not possible to find significant differences in the IVF programme with regard to stimulation outcome between Gonal-F and Puregon. The results of this study indicate that Gonal-F and Puregon may be equally suitable for use in ovarian stimulation for IVF.

Key words: FSH/IVF/oestradiol/ovary/progesterone

Introduction

Controlled ovarian hyperstimulation for in-vitro fertilization (IVF) using preparations containing follicle stimulating hormone (FSH) has been routinely performed since the 1980s. The early preparations used were urinary human menopausal gonadotrophins (HMGs) containing FSH and luteinizing hormone (LH) in a 1:1 ratio (Muasher *et al.*, 1985; Navot and Rosenwaks, 1988; Palermo *et al.*, 1988; Edelstein *et al.*, 1990; Torok *et al.*, 1991). In the 1990s, highly purified urinary FSH preparations were introduced because of a desire to provide drugs for s.c. injections and with a lower risk of allergic reactions (Howles *et al.*, 1994). Expectations of future insufficient supplies of urine as a raw material to meet the demands of the increased use of FSH also made it necessary to find other sources than urine. Intensive research resulted in recombinant FSH, and in 1992, the first babies were born following treatment with FSH produced in that way (Devroey *et al.*, 1992; Germond *et al.*, 1992).

Since the autumn of 1996, two recombinant FSH preparations (Gonal-F and Puregon) have successively been introduced in IVF markets starting in Europe, and we now have experience of the two preparations available in Sweden for nearly 1 year. Both preparations had undergone extensive clinical trials in collaboration with several European centres (Loumaye *et al.*, 1993; Devroey *et al.*, 1994), and at the time of the introduction of the products in Sweden, it was emphasized that the two preparations were similar but not identical.

Data exist to suggest that recombinant FSH is more potent than the highly purified urinary FSH preparations (Out *et al.*, 1996, 1997). Partly based on these studies, Puregon was believed to be somewhat more potent than Gonal-F; therefore, the drug was made available in ampoules of 50, 100 and 150 IU rather than the 'standard' size of 75 and 150 IU. Furthermore, recommended dosages were slightly lower than for Gonal-F.

Both preparations have been used at our clinic since the beginning of 1997. It was postulated that there might be a difference between the two preparations with regard to stimulation outcome, and therefore the present analysis of stimulation outcome in 296 consecutive cycles in 1997 was performed.

Materials and methods

Study population

The study population consisted of 218 patients treated at our clinic in 1997, and encompasses a total of 296 cycles. Gonal-F was given to 145 patients in 199 cycles, and Puregon was given to 73 patients in 97 cycles. Puregon was made available later than Gonal-F in Sweden, which is why a larger number of patients were given Gonal-F. As soon as both preparations were available, patients were assigned either Gonal-F or Puregon based on the odd or even last digit in their date of birth (year, month, day). Characteristics of the patients in the study are given in Table I.

Ovarian stimulation protocol

All patients followed our standard long gonadotrophin-releasing hormone agonist (GnRHa)-HMG protocol for stimulation as described earlier (Csemiczky *et al.*, 1995; Fried *et al.*, 1996). GnRHa (Suprefact; Svenska Hoechst AB, Stockholm, Sweden) was administered as a nasal spray 6×200 μg/day starting on day 21 of the menstrual cycle, and was reduced to half the dose when FSH injections were commenced. After down-regulation was verified by vaginal ultrasound scanning, 75–300 IU/day of recombinant FSH (Gonal-F; Serono

Table I. Characteristics of patients in the study. Values given as means ± SD or in %

	Gonal-F (n = 145)	Puregon (n = 73)
Age (years)	33.3 ± 0.3 (range 22–39)	33.3 ± 0.3 (range 26–39)
BMI (kg /m^2)	23.0 ± 0.3	22.4 ± 0.4
Total dose FSH (IU)	2182 ± 61	2105 ± 74
Length of stimulation (days)	15.2 ± 0.2	16.0 ± 0.4
Indication for treatment (%)		
Tubal factor	31.0	26.0
Male factor	15.2	16.4
Mixed/unexplained	53.8	57.5

BMI = body mass index; FSH = follicle stimulating hormone.

Nordic AB, Sollentuna, Sweden; Puregon; Organon Sweden AB, Göteborg, Sweden) was administered s.c.

Starting dose was adjusted according to the same rules in both groups. The initial dose of FSH was generally 150 IU up to age 35 years, and 225 IU above this age, unless response to previous FSH stimulation at IVF indicated otherwise. In four cases in each group with the diagnosis of polycystic ovaries and a known tendency for strong response to FSH stimulation, the starting dose was 75–100 IU. In 13 Gonal-F cycles and five Puregon cycles, previously known low responders were given 300 IU FSH. In 12 Gonal-F and 10 Puregon cycles, the dose was raised by 50–75 IU after 7–8 days of stimulation due to poor ovarian response.

Follicular development and endometrial growth were monitored by vaginal ultrasonography using a Siemens Sonoline SI/200 in combination with blood samples for 17β-oestradiol assays. When an adequate stimulation was achieved, i.e. a controlled rise in serum oestradiol and a leading follicle diameter of at least 17 mm, 10 000 IU human chorionic gonadotropin (HCG; Profasi; Serono Nordic AB, Sollentuna, Sweden) was given s.c. Approximately 35 h later ovum retrieval was performed by transvaginal ultrasound-guided follicle aspiration. IVF, embryo transfer and pregnancy follow-up was performed as described elsewhere (Csemiczky *et al.*, 1995). Luteal phase support was given using 400 mg micronized progesterone as vaginal suppositories three times daily until a pregnancy test was performed and if found positive, continued 8 weeks after embryo transfer. Pregnancy was defined as a serum HCG concentration >10 IU/l 2 weeks after embryo transfer and subsequently rising. Clinical pregnancy was defined as presence of an intrauterine fetus with regular heart beats. Criteria for cycle cancellation were (i) no rise in oestradiol even after an increase in dose, (ii) only three or fewer follicles approaching 17 mm diameter, (iii) high risk for developing ovarian hyperstimulation syndrome – sharp rise in oestradiol and >25 follicles.

Hormone assays

Serum 17β-oestradiol and progesterone were assayed by the Central Laboratory for Clinical Chemistry, Karolinska Sjukhuset by radioimmunoassay, using reagents from the Farmos Group (Oulu, Finland).

Statistical analysis

Prior to statistical analysis, data were tested for Gaussian distribution using a normality test (the Kolmogorov–Smirnov test). Some data were found not to be normally distributed. Therefore, non-parametric statistics were used for all the data. Comparisons between mean values were made using the Mann–Whitney rank sum test. Although the standard errors were therefore not used for statistical comparisons, they have been appended to the mean values in the tables. Correlation analysis was performed using Spearman correlation. Tests were performed with the statistical package Graphpad Prism and Statmate (Graphpad Software Inc, San Diego, CA, USA). A P-value < 0.05 was considered significant.

Table II. Outcome after stimulation with two preparations of recombinant FSH. Values in brackets are percentages

	Gonal-F	Puregon
No. of cycles started in each group	199 (100)	97 (100)
No. of cycles with HCG and oocyte retrieval	184 (92.5)	87 (89.7)
No. of cycles with embryo transfer	170 (85.4)	80 (82.5)
No. of cycles with clinical pregnancy	58 (29.1)	28 (28.9)
No. of cycles with ongoing pregnancy	41 (20.6)	20 (20.6)

HCG = human chorionic gonadotrophin.

Table III. Percentage of pregnancies obtained using the two preparations of recombinant FSH in relation to number of oocyte retrieval and embryo transfer cycles

Preparation	Oocyte retrieval cycles	Embryo transfer cycles
Gonal-F	31.5 (58/184)	34.1 (58/170)
Puregon	32.2 (28/87)	35.0 (28/80)

Table IV. Number of pregnancies in relation to number of cycles using either of the two preparations of recombinant FSH. Figures in brackets denote number of pregnancies

Number of cycles	Gonal-F	Puregon
1	107 (44)	59 (23)
2	37 (13)	14 (5)
3	1 (1)	–

Results

The characteristics of the study population are presented in Table I. There were no significant differences between the Gonal-F and Puregon groups with regard to age, reasons for infertility, duration of stimulation or presence of male factor. Regarding male factor, most of the male partners had sperm counts $>5\times10^6$/ml after swim-up preparation. However, 29 males (15.8%) in the Gonal-F group and 17 (19.5%) in the Puregon group had sperm counts $<1\times10^6$/ml. In 22 and 12 of these cases respectively, intracytoplasmic sperm injection (ICSI) was performed. There was no difference in the use of ICSI between the two groups. For the remaining patients, regular IVF was used. Cryopreservation was performed in eight cycles (seven Gonal-F, one Puregon) with a mean of 4.4 (three to six) preimplantation embryos. In five Gonal-F freeze cycles embryo transfer was performed, resulting in one twin pregnancy, whereas the embryo transfer in the Puregon freeze cycle was unsuccessful.

The outcome of stimulation of the two groups is shown in Tables II–IV. The pregnancy rate (calculated on the number of cycles where embryo transfer was performed) was not

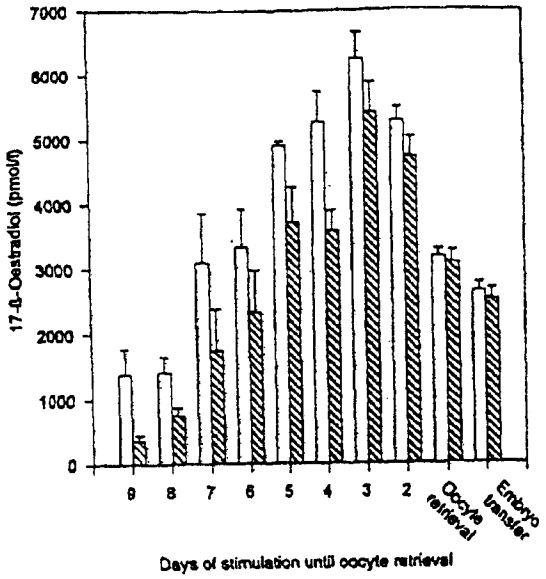

Figure 1. Serum oestradiol concentrations (mean ± SEM) during cycles stimulated with either Gonal-F (open bars) or Puregon (hatched bars). Concentrations were measured daily from 9 days before oocyte retrieval until 2 days before retrieval, on the day of oocyte retrieval and on the day of embryo transfer.

Table V. Stimulation response, number of replaced embryos and sperm quality in 271 consecutive cycles where HCG was given and where oocyte retrieval was performed, using two different preparations of recombinant FSH. Values given as means ± SEM

	Gonal-F (n = 184)	Puregon (n = 87)
Endometrial thickness before oocyte retrieval (mm)	10.9 ± 0.2	11.2 ± 0.3
Follicles 12–15 mm (n)	7.3 ± 0.5	6.5 ± 0.6
Follicles >15 mm (n)	7.8 ± 0.5	6.2 ± 0.5
Oocytes retrieved (n)	7.9 ± 0.3	7.4 ± 0.4
Ooocytes fertilized (n)	4.8 ± 0.2	4.0 ± 0.3
Oocytes cleaved (n)	4.7 ± 0.2	3.9 ± 0.3
Sperm count (10^6/ml)	6.1 ± 0.3	5.2 ± 0.4
Spermatozoa with good motility (%)	94.8 ± 0.9	94.7 ± 1.3
Pre-embryos replaced (n) (170/80)	1.8 ± 0.04	1.8 ± 0.06

significantly different between the two groups, resulting in clinical pregnancy rates of 29.1 and 28.9% for Gonal-F and Puregon respectively, whereas the ongoing pregnancy rates were 20.6% for both groups. The biochemical pregnancy rates were 34.1 and 35.0% per embryo transfer for Gonal F and Puregon respectively.

There was no difference between the two groups in the ovarian and endometrial response to the FSH stimulation (Table V). The endometrial thickness before oocyte retrieval, as well as the numbers of smaller (12–15 mm) and larger (>15 mm) follicles, were similar. Neither were there any significant differences with regard to the number of oocytes retrieved, fertilized, cleaved and replaced, nor in sperm counts/sperm progressive motility. There was a higher number of larger follicles in the Gonal-F group, but the difference was not statistically significant (P = 0.06).

The ovarian response in terms of oestradiol production/secretion is shown in Figure 1. Oestradiol levels in the early follicular phase were slightly higher in Gonal-F than in the Puregon cases, however, the difference was not significant up to 3 days before oocyte retrieval at oocyte retrieval or at embryo transfer. Oestradiol concentrations were compared in patients becoming pregnant and those remaining non-pregnant. Neither in the Gonal-F nor in the Puregon-treated group were the oestradiol levels at oocyte retrieval or at embryo transfer significantly different in this respect.

The progesterone levels in serum at oocyte retrieval, i.e. the day after HCG, were significantly higher in the Gonal-F group compared to the Puregon group, 37.7 ± 1.5 nmol/l (n = 164) versus 30.3 ± 1.8 nmol/l (n =76) (P = 0.005). In the patients who became pregnant, the corresponding values were 35.4 ± 2.8 nmol/l (n = 52, Gonal-F) versus 26.7 ± 3.3 nmol/l (n = 24, Puregon) (P = 0.03), and in the non-pregnant patients the values were 38.7 ± 1.8 nmol/l (n = 112, Gonal-F) versus 32.0 ± 2.1 nmol/l (n = 47, Puregon) (P = 0.04). No differences in progesterone concentrations were seen between the two groups or between pregnant and non-pregnant patients on the day of embryo transfer.

A correlation analysis regarding progesterone concentrations at oocyte retrieval revealed that in both the Gonal-F and the Puregon group, progesterone concentrations were strongly correlated with oestradiol levels from 2 days prior to oocyte retrieval, on the day of oocyte retrieval and on the day of embryo transfer (P < 0.0001). In addition, progesterone concentrations were also correlated with the number of small follicles (12–15 mm) (P = 0.03), the total number of oocytes retrieved (P = 0.005) and the number of cleaved oocytes (P < 0.0001).

A detailed analysis was performed regarding the total dose of FSH given in the two groups, in relation to pregnancy rate, duration of stimulation, oestradiol and progesterone at oocyte retrieval, number of smaller and larger follicles and number of oocytes retrieved and fertilized (Figure 2a–h). Patients given either Gonal-F or Puregon were assigned to five arbitrarily designed groups on the basis of the total dose of FSH given; ≤1500, 1501–2000, 2001–2500, 2501–3000 and >3000 IU. No significant differences were found in pregnancy rate (Figure 2a) or in any of the other examined parameters (Figure 2b–h) in relation to total dose between the administered preparations used for stimulation.

The duration of stimulation was correlated to total dose within both the Gonal-F and the Puregon group, increasing with higher doses (Figure 2b). At a total dose of ≤1500 IU, oocyte retrieval was performed at 13.4 ± 0.5 days in the Gonal-F group as compared to 14.6 ± 0.3 in the Puregon group. At a total dose of >3000 IU, the duration of the stimulation increased to 17.3 ± 0.5 and 16.5 ± 0.4 days respectively.

The serum concentrations of oestradiol at oocyte retrieval were also correlated to the total dose in both the Gonal-F and the Puregon group, and decreased with higher doses (Figure 2c). At a total dose of ≤1500 IU, oestradiol was 3808 ± 283

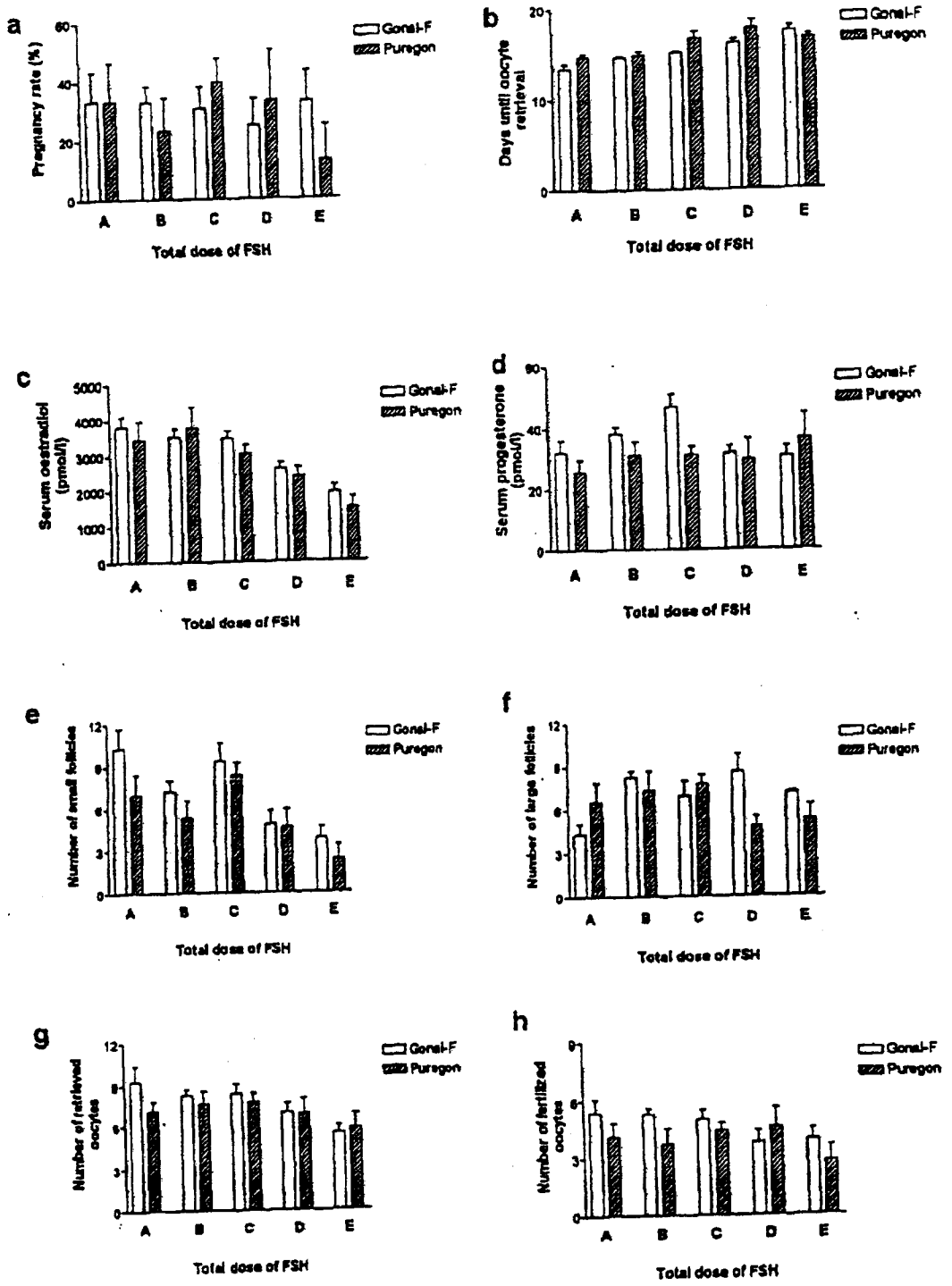

Figure 2. Total dose of follicle stimulating hormone (FSH) given in cycles stimulated with either Gonal-F (open bars) or Puregon (hatched bars) (mean ± SEM) in relation to (a) pregnancy rate, (b) length of stimulation, (c) oestradiol at oocyte retrieval, (d) progesterone concentrations at oocyte retrieval, (e) number of smaller (12–15 mm) follicles, (f) number of large (>15 mm) follicles, (g) number of retrieved oocytes and (h) number of fertilized oocytes. Patients given either Gonal-F or Puregon were assigned to five arbitrarily designed groups (A–E) on the basis of the total dose of IU of FSH given: A, <1500; B, 1501–2000; C, 2001–2500; D, 2501–3000; and E, >3000 IU. The number of patients in each group was for Gonal-F/Puregon: A: 21/15, B: 73/18, C: 42/37, D: 24/9, E: 24/8.

pmol/l in the Gonal-F group as compared to 3459 ± 509 pmol/l in the Puregon group. The corresponding concentrations for a total dose of >3000 IU were 1928 ± 212 and 1508 ± 301 pmol/l. The serum concentrations of progesterone at oocyte retrieval were higher in the Gonal-F group at total doses up to 2500 IU (Figure 2d), but not above.

The numbers of smaller follicles (12–15 mm) were related to the total dose in both groups, decreasing with higher doses (Figure 2e). At ≤1500 IU, there were 10.2 ± 1.4 follicles in the Gonal-F group as compared to 7.3 ± 1.4 in the Puregon group. At >3000 IU, the corresponding numbers were 3.8 ± 0.8 and 2.3 ± 1.0 respectively. The numbers of larger follicles (>15 mm) did not vary significantly with total dose (Figure 2f).

The numbers of retrieved and fertilized oocytes were also correlated to the total dose in both groups, and decreased with a higher dose (Figure 2g, h). At ≤1500 IU, there were 9.3 ± 1.1 and 5.4 ± 0.8 oocytes respectively, in the Gonal-F group as compared to 7.2 ± 0.7 and 4.1 ± 0.7 oocytes in the Puregon group. At >3000 IU, the corresponding numbers were 5.4 ± 0.6 and 3.9 ± 0.6 respectively in the Gonal-F group as compared to 5.8 ± 1.1 and 2.8 ± 0.8 in the Puregon group.

Discussion

The present study clearly indicates that both of the recombinant FSH preparations currently on the market are equally well suitable for use in ovarian stimulation. Both can be self-administered s.c., which is a great clinical advantage. The results of this study reveal no significant clinical difference between Gonal-F and Puregon regarding number of follicles stimulated, number of oocytes retrieved and fertilized or in pregnancy rate. A recent comparison between Gonal-F and Puregon on a small number of patients, 22 in each group (Brinsden *et al.*, 1998), is in agreement with the results presented here. The current study was designed as a prospective randomized study in 1997, but due to the later introduction of Puregon, there was a higher number of Gonal-F cycles. Even though this is a drawback, the two groups were well matched with regard to age, body mass index and indication for treatment (see Table I).

The only statistical difference found was that progesterone on the day of oocyte retrieval was higher in the Gonal-F group. A slightly higher number of follicles and higher levels of oestradiol in the Gonal-F group were also observed, although these were not statistically significant on the day of oocyte retrieval. The higher progesterone concentrations observed in the Gonal-F group may be related to this observation. The correlation of progesterone concentrations on the day of oocyte retrieval to the oestradiol concentrations, to the number of smaller follicles and to the number of oocytes retrieved strongly supports this suggestion. The recruitment of a higher number of follicles using Gonal-F would explain both higher oestradiol and progesterone concentrations. This is also supported by findings in a recent study comparing the efficacy of 100 IU versus 200 IU Puregon, where it was found that the 200 IU group had significantly higher progesterone concentrations on the day of HCG; this group also had more follicles and more oocytes retrieved (Out *et al.*, 1999). In the current study, the Gonal-F group received a slightly higher total dose of FSH than the Puregon group, 2182 ± 61 as compared with 2105 ± 74, a difference of 77 IU. This difference may be related to the higher numbers of follicles in the Gonal-F group, but as mentioned above, neither the difference in total dose, nor the difference in follicle numbers was statistically significant.

The higher concentrations of progesterone found in the Gonal-F group did not appear to have any clinically relevant effects as compared to Puregon, i.e. the pregnancy rates were the same, as well as the proportion of biochemical pregnancies and miscarriages. Elevated progesterone concentrations in the follicular phase have been suggested to have a predictive value for the outcome of pregnancies achieved by IVF by some authors (Burns *et al.*, 1994), but this has been questioned by others (Huang, 1996). In the present study, a difference was only seen on the day of oocyte retrieval, and as discussed above, may be related to the number of developing follicles. On the day of embryo transfer there was no significant difference between the Gonal-F group and the Puregon group, indicating that there is no difference between the two in the luteal phase.

The small difference observed between Gonal-F and Puregon may be related to differences in molecular composition of the two preparations. It has been shown that the clinical efficacy of FSH principally may be related to the proportion and amount of acidic isoforms and to the degree of molecular complexity (Chappel, 1995). In a recent biochemical comparison between Gonal-F and Puregon, it was shown that Gonal-F is slightly more acidic than Puregon, whereas both Gonal-F and Puregon were far more similar to human urinary menopausal gonadotrophins (Fertinorm/Metrodin) than to highly purified urinary FSH (Fertinorm HP/Metrodin HP). Both recombinant FSH preparations, in addition, were shown to be more similar to human mid-cycle endogenous FSH than the urinary preparations (Robertson, 1998; see also Recombinant Human FSH Product Development Group, 1998). Earlier studies on human menopausal gonadotrophin preparations from these two suppliers have also indicated differences in molecular composition using isoelectric focusing (Harlin *et al.*, 1986).

The analysis regarding stimulation outcome in relation to the total dose given revealed no difference in potency between Gonal-F and Puregon. The two preparations required a similar stimulation length for optimal effect, 15.2 days for Gonal-F and 16 days for Puregon (given as days from start of FSH injections until oocyte retrieval, in terms of days of FSH-injections this translates to 12.2 days for Gonal-F and 13 days for Puregon). It has been claimed that recombinant FSH is more effective than highly purified urinary FSH (Bergh *et al.*, 1997; Out, 1996; Recombinant Human FSH Product Development Group, 1998). Recombinant FSH in this respect may be more similar to the original HMG preparations. It should however be noted that duration of stimulation and total dose required may be dependent on the type of down-regulation used, why direct comparisons between studies with different protocols are difficult to evaluate. Even when a common protocol was used, variation between clinics was identified as an important factor potentially contributing to observed differences in the five-centre prospective, randomized, double-blind clinical trial of two fixed doses of Puregon (Out *et al.*, 1999). In a previous study, HMG (Pergonal) was compared with highly purified FSH (Fertinorm-HP/Metrodin) using the same type of down-regulation used in the present study, and

it was observed that stimulation length as defined above was 13.9 days for Pergonal and 14.3 days for Fertinorm-HP (Fried *et al.*, 1996).

In conclusion, this study shows that the two available recombinant FSH preparations for ovarian stimulation in IVF are equally well suited, and both fulfil the essential requirements for acceptable pregnancy rates.

Acknowledgements

This study was supported by Swedish Medical Research Council (14X-07614) and Karolinska Institutets Fonder. We thank all staff at the Reproductive Medical Center, particularly Marita Johansson, Sylvia Johansson, Gunilla Landberg and Laila Einarsson for their contribution to this work.

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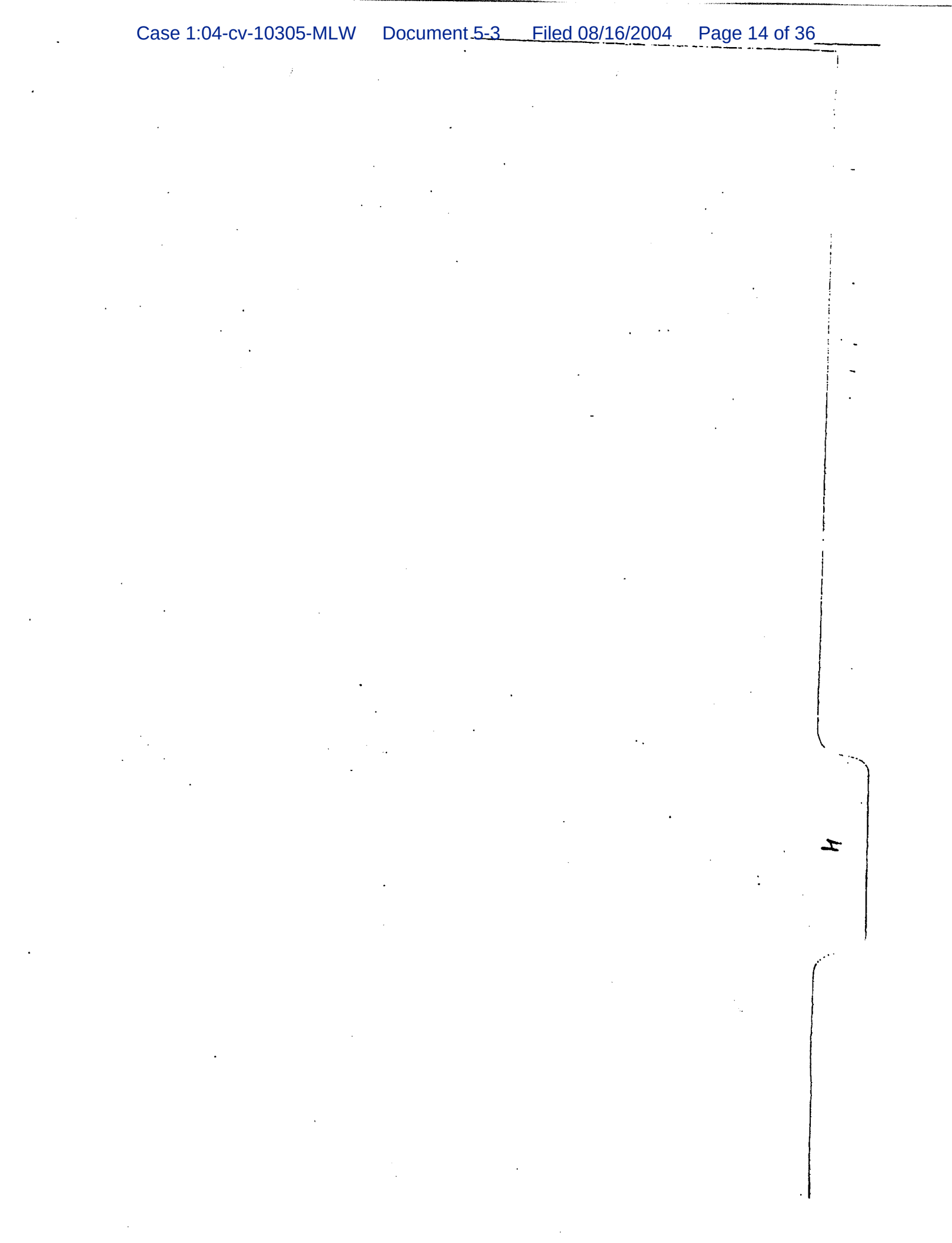

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A comparison of the efficacy and tolerability of two recombinant human follicle-stimulating hormone preparations in patients undergoing in vitro fertilization-embryo transfer

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Objective: To compare the efficacy and tolerability of two recombinant human FSH (r-hFSH) preparations, follitropin-α (Gonal-F; Ares Serono, Geneva, Switzerland) and follitropin-β (Puregon; Organon, Oss, the Netherlands), for superovulation in patients undergoing IVF-ET.

Design: Randomized, parallel-group, assessor-blind, single-center trial.

Setting: Outpatient tertiary referral center for assisted reproductive techniques.

Patient(s): Forty-four infertile women undergoing IVF-ET.

Intervention(s): After down-regulation with buserelin acetate, patients were randomized to receive follitropin-α or follitropin-β, 150 IU/d for 6 days; after that, dosages were adjusted according to the ovarian response.

Main Outcome Measure(s): Cumulative dose of r-hFSH; duration of r-hFSH treatment; number of follicles of ≥11 mm and of 14 mm on day 7 of r-hFSH treatment and on the day of hCG administration; number of oocytes retrieved; number of viable embryos; and number of pregnancies (biochemical, ectopic, miscarried) and clinical pregnancies.

Result(s): There were no statistically significant differences in any efficacy measures between the two preparations. The incidence of systemic adverse events was comparable in the two groups. Local reactions at the injection site were significantly more common and more severe with follitropin-β than with follitropin-α

Conclusion(s): Follitropin-α and follitropin-β have comparable efficacy in patients undergoing IVF-ET. (Fertil Steril® 2000;73:114–6. ©1999 by American Society for Reproductive Medicine.)

Key Words: Recombinant human follicle-stimulating hormone, r-hFSH, follitropin-α, follitropin-β, in vitro fertilization

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Ovarian stimulation with FSH plays a key role in IVF-ET as it increases the number of oocytes produced and hence the chance of a successful outcome of treatment. Until recently, FSH was extracted from the urine of postmenopausal women. The consistency of the product was variable. The more recently developed recombinant human FSH (r-hFSH), produced from CHO cells (1), has the advantage of high purity, high specific activity, and consistent composition (2).

Two r-hFSH preparations currently are available for clinical use, follitropin-α (Gonal-F; Ares Serono, Geneva, Switzerland) and follitropin-β (Puregon; Organon, Oss, the Netherlands); both previously have been compared with urinary FSH preparations (3–5).

This article presents the first direct comparison of the efficacy and tolerability of follitropin-α and follitropin-β in women undergoing ovarian stimulation for IVF-ET.

MATERIALS AND METHODS

The protocol for this prospective, randomized, single-center, assessor-blind, parallel-group

TABLE 1

Outcome of oocyte retrieval and ET in 44 patients who received r-hFSH, 34 of whom underwent ET.

Variable	Treatment		P value*
	Follitropin-α	Follitropin-β	
Cumulative dose of r-hFSH (IU)	1,332.95 ± 513.90	1,332.95 ± 347.9	1.00
Duration of treatment with r-hFSH (d)	8.50 ± 0.91	8.68 ± 1.29	.59
No. of oocytes retrieved	12.1 ± 7.9	12.3 ± 8.6	.94
No. of follicles of ≥14 mm on day of hCG administration	10.1 ± 5.8	10.7 ± 5.5	.71
Fertilization rate (%)	52.9 ± 0.3	53.5 ± 0.3	.94
No. of embryos transferred	2.2 ± 0.5	2.4 ± 0.7	.2
No. of embryos cryopreserved	3.91 ± 5.76	3.32 ± 5.47	.72
No. of pregnancies (%)	7 (31.8)	6 (27.3)	.74†
No. of clinical pregnancies (%)	7 (31.8)	4 (18.2)	.30†
Embryo implantation rate (%)	19.60 ± 0.30	17.60 ± 0.35	.86
No. of local reactions at injection site by severity (%)			
None	156 (83.4)	131 (72)	<.05
Mild	27 (14.4)	37 (20.3)	
Moderate	4 (2.1)	11 (6)	
Severe	0 (0)	3 (1.7)	

Note: Values are means ± SD unless otherwise indicated.
* Determined by analysis of variance/analysis of covariance.
† According to a logistic regression model.
Brinsden. IVF-ET. Fertil Steril 2000.

study proposed the enrollment of 40 infertile women (20 in each treatment group) aged 18–38 years for whom IVF-ET was indicated. The study was performed according to the principles of the Declaration of Helsinki and good clinical practice; the Bourn Hall Clinic Ethics Committee gave its approval, and all patients provided written informed consent. All patients underwent pituitary down-regulation with buserelin acetate, 0.2 mg/d SC, before the start of r-hFSH treatment. Down-regulation was confirmed by ultrasound examination and a serum E_2 concentration of <50 pg/mL at least 10 days after the start of buserelin acetate treatment.

Patients were randomized to receive either follitropin-α or follitropin-β, 150 IU/d SC for 6 days; after that, the dosage was adjusted according to the ovarian response. Urinary hCG (10,000 IU, Profasi; Ares Serono) was given by SC injection when the patient had at least one follicle of ≥18 mm in diameter and two follicles of ≥16 mm in diameter measured by vaginal ultrasound examination.

The response to ovarian stimulation was monitored by ultrasound examinations and serum E_2 level determinations. After oocyte retrieval, the number and maturity of the oocytes were recorded. The fertilization rate and the number and quality of the embryos were recorded on the following days. A diagnosis of clinical pregnancy was confirmed by measurement of serial serum β-hCG concentrations and visualization of a fetal sac on subsequent ultrasound examination.

All adverse events were recorded by severity throughout the study, and injection site reactions during r-hFSH treatment were recorded by the patients on diary cards. Symptoms of itching, swelling, redness, bruising, and pain were classified as absent, mild, moderate, or severe, and their duration was noted.

RESULTS

The two groups of 22 patients each were comparable with respect to their demographic characteristics and their gynecologic and obstetric histories. Oocytes were retrieved from all 44 patients who received r-hFSH, and 34 of the patients underwent ET. Of the 10 patients who did not undergo ET, 1 in each group had oocytes that did not become fertilized, 7 had all their embryos frozen in accordance with the center's policy on risk for ovarian hyperstimulation syndrome (3 in the follitropin-α group and 4 in the follitropin-β group), and 1 had an adverse event (metrorrhagia on the day of ET). There were no statistically significant differences in any of the outcome measures between the two groups (Table 1).

There were no serious adverse events. There were 56 mild or moderate systemic adverse events, 21 in 11 patients who received follitropin-α and 35 in 14 patients who received follitropin-β. The principal adverse events, including ovarian hyperstimulation, occurred in no more than 3 patients in each group. The incidence of these events did not differ significantly between the groups.

Twelve (57.1%) of 21 patients who received follitropin-β and 8 (36.4%) of 22 patients who received follitropin-α reported at least one symptom ($P=.049$). No patient who received an injection of follitropin-α had a local reaction

recorded as severe, whereas 3 patients who received follitropin-β reported severe reactions to injections. The incidence of local reactions at the r-hFSH injection site is reported in Table 1.

DISCUSSION

The first direct comparison of the two available r-hFSH preparations demonstrated their equivalent efficacy, with no statistically significant differences in any of the measured outcomes. This study also further confirmed the previously documented high efficacy of r-hFSH (2–5) as indicated by the low cumulative dose of FSH (equivalent to 18 75-IU ampules of FSH) and the large number of oocytes retrieved. Both preparations were well tolerated. The most common adverse event, ovarian hyperstimulation, occurred in three patients who were given follitropin-α and two patients who were given follitropin-β. There were statistically significant differences in the incidence, severity, and duration of local reactions at the injection site.

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5

Plus 5

SEP 13 1994

NDA20-378
Gonal-F

Serono Laboratories, Inc.
July 11, 1994

Medical Officer's Comments Regarding Statistical Review and Evaluation Dated June 30, 1994

Our statistician discussed three relevant issues in his review and evaluation:

1. A significant treatment difference was detected in favor of Metrodin over Gonal-F with regard to the primary efficacy parameter.

2. The resulting 95% primary efficacy parameter treatment difference confidence interval indicates that it is statistically conceivable that a clinically important difference exists in favor of Metrodin over Gonal-F.

3. The severity of redness developed as a consequence of treatment injections was significantly greater with Gonal-F than with Metrodin.

Pertinent comments addressed by him included the following:

1. Study GF5503 was adequately powered to detect a treatment difference of 2 matured follicles where a difference of 2 follicles or less was not considered clinically important.

2. The sponsor's all patient primary efficacy parameter (matured follicles) analysis detected a significant difference (p=.0365) in favor of Metrodin over Gonal-F. In fact, the 95% between-treatment (Metrodin minus Gonal-F) confidence interval (.09, 2.61) contains values in excess of the aforementioned critical threshold value of 2 matured follicles. Consequently, by the sponsor's definition, it is statistically conceivable that a clinically important treatment difference with regard to matured follicles exists in favor of Metrodin over Gonal-F.

3. A numerical superiority was also detected in favor of Metrodin over Gonal-F with regard to the sponsor's "relevant" secondary efficacy parameters.

4. In addition, local injection reactions exhibited by study patients indicated that such reactions were more severe in the Gonal-F treatment group with statistical significance (p=.043) being attained with regard to the severity of redness.

5. Consequently, given the above mentioned safety and efficacy results, the clinical relevance of the sponsor's claim that "clinical Study GF5503 has demonstrated that Serono's recombinant human FSH (Gonal-F) is as safe and effective as urinary FSH for the stimulation of follicular development" should be evaluated by the clinical reviewer.

A clinical difference of significance in the primary efficacy end point of number of mature follicles was deemed to be a mean difference of 2 or more follicles between treatment groups. This end-point was chosen since follicle growth represents a measurable effect of FSH in its specific target organ. Based on an earlier study using Metrodin where the mean number of follicles achieved at mid-cycle was 7.6 and the standard deviation was 3.7, it was estimated that 106 evaluable patients would be required to give 80% power to detect a mean difference of two or more follicles ≥ 14mm between the two treatment groups at a two-sided significance level of 0.05.

The sponsor calculated the 95% confidence intervals of the difference between Metrodin and Gonal-F taking into account the center effect for the number of follicles ≥ 14mm in diameter (i.e. number of mature follicles) as well as the relevant secondary efficacy parameters for both the "All Patients" and the "Evaluable Patients" analyses. Considering the primary efficacy end-point of Study GF5503 (number of follicles ≥ 14mm), the actual mean difference, which is the best estimate of the true difference, was 1.4 follicles. This difference represents around one sixth of the actual mean number of follicles ≥ 14mm obtained with Gonal-F (8.3.) or Metrodin (9.7) taking into account the center effect. The 95% CI of this difference, however, indicates that a difference of up to approximately 3 follicles cannot be excluded (as well as almost no difference), but a mean difference of 1.4 follicles is not clinically significant.

Even the worst case (i.e. 3 follicles of mean difference) is not expected to have a meaningful consequence on treatment outcome since the mean difference tends to be reduced in the subsequent steps of the IVF-ET treatment and in these therapeutic procedures, to reduce the incidence of multiple pregnancies, it is usually not recommended to replace more than three embryos. It has always been my opinion that the endpoint of practical clinical importance for in-vitro fertilization studies is the live birth rate and not the number of oocytes recovered or fertilized or the number of embryos cleaved. In this study, both pregnancy and live birth rates were similar in the two treatment groups despite the difference in the mean number of follicles ≥ 14mm. As pointed out in my original review, a difference of 1.4 in the mean number of follicles ≥ 14mm in diameter was statistically significant, but was not considered to be clinically important.

I agree that the severity of redness developed as a consequence of treatment injections was significantly greater with Gonal-F than with Metrodin. This is stated in my original review. This is not unexpected since the Gonal-F was administered subcutaneously in the abdominal wall and the Metrodin was administered intramuscularly in the buttocks. All of the local injection reaction mentioned in Table 3 of the Statistical Review and their frequency are mentioned in the ADVERSE REACTIONS section of the submitted draft labeling.

Ridgely C. Bennett

Ridgely C. Bennett, M.D., M.P.H.

cc: NDA 20-378
HFD-510/Bennett/Corfman/rd/dj/7.12.94/ft/dj/7.15.94
HFD-510/Pauls
HFD-510

P. Pauls 7-15-94

MAR 3 1994

MEDICAL OFFICER'S ORIGINAL SUMMARY OF NDA 20-378

SPONSOR'S NAME: Serono Laboratories, Inc.

PROPOSED TRADE NAME: Gonal-F

GENERIC NAME: Recombinant Human Follicle Stimulating Hormone.

ACTIVE INGREDIENT: Follicle Stimulating Hormone.

DOSAGE FORM: Sterile, lyophilized form.

STRENGTHS: Each single dose vial contains 75 or 150 I.U. of FSH activity.

PRESCRIPTION OR O.T.C.: Prescription

I. Proposed Uses:
   A. Stimulation of follicular development in patients with polycystic ovarian syndrome who have an elevated LH/FSH ratio and who have failed to respond to adequate clomiphene citrate therapy.
   B. Stimulation of multiple follicular development in ovulatory patients undergoing Assisted Reproductive Technologies such as in vitro fertilization.

II. Initial Dosage Recommended:
   A. Polycystic Ovarian Syndrome: 75 I.U. for 7-12 days.
   B. Assisted Reproductive Technologies: 150 I.U. per day.

III. Same or Related Drug: Metrodin (urinary human follicle stimulating hormone).

IV. Pharmacology: Refer to pharmacologist's review.

V. Pharmacodynamics: Refer to pharmacologist's review.

VI. Introduction:
   GONAL-F is a preparation of the human gonadotropin FSH (follicle stimulating hormone) produced by genetic engineering in Chinese Hamster Ovary (CHO) cells and purified by a process which includes immunoaffinity chromatography using a murine-derived monoclonal antibody to FSH. The resulting product has a high specific activity of ≥7,000 IU FSH/mg protein. The finished product will be available as a lyophilized powder containing 75 IU and 150 IU for either subcutaneous or intramuscular injection.

The purity of GONAL-F and complete absence of contaminating LH or other urinary proteins will make available to physicians and patients for the first time a highly purified, monotherapeutic human gonadotropin product. Elimination of the urinary source also enhances safety.

The therapeutic property of FSH in women has already been well-documented in Metrodin clinical studies. FSH stimulates ovarian follicular development and is applied to two clinical situations:

1. The stimulation of multiple follicular development in ovulatory patients undergoing assisted reproductive technologies such as in vitro fertilization, and
2. The stimulation of follicular development in patients with polycystic ovarian syndrome (PCO) who have an elevated LH/FSH ratio and who have failed to respond to adequate clomiphene citrate therapy.

To confirm the efficacy of FSH in GONAL-F, IVF was chosen as a model because it allows a comparative evaluation of more variables, such as oocyte quality, fertilization rate, early embryonic development and embryo implantation rate in addition to number of follicles, pregnancy rate and pregnancy outcome. Also, since standard IVF-ET treatments are currently performed with a gonadotropin-releasing hormone (GnRH) agonist which reduces endogenous secretion of both luteinizing hormone (LH) and FSH to levels lower than in hypothalamic-pituitary dysfunction, this model enables assessment of FSH therapy in the absence of endogenous gonadotropin secretion, a most demanding clinical situation.

It is proposed that GONAL-F be approved for the same indications as currently indicated for Metrodin for the following reasons:

1. GONAL-F and Metrodin are therapeutic preparations of the human gonadotropin FSH having similar physiochemical, immunological, and biological properties.
2. GONAL-F and Metrodin have similar pharmacokinetics and pharmacodynamics in vivo.
3. Equivalent efficacy and safety has been demonstrated between GONAL-F and Metrodin in a comparative, adequate and well-controlled clinical study (Study GF 5503).
4. A satisfactory safety profile has been established in a total of 232 patients treated with GONAL-F in twelve clinical studies, three of which are completed and nine are ongoing.
5. The approval of GONAL-F for both indications allow GONAL-

F to replace Metrodin in the marketplace, eliminating a urinary sourced product that is only about 3% pure.

Prior to clinical evaluation, an assessment of GONAL-F™ showed it to be similar to urinary hFSH in terms of physiochemical characteristics, receptor binding affinity, pharmacodynamic properties in rats and monkeys, toxicity profile, pharmacokinetic and pharmacodynamic characteristics in healthy human volunteers. Therefore, the aim of the clinical evaluation of GONAL-F™ was to investigate whether GONAL-F™ is as effective and safe in treating female patients as a reference drug, Metrodin® (a registered and widely marketed preparation of native urinary hFSH).

VII. Clinical Pharmacology: Refer to pharmacokineticist's review for a summary of human pharmacokinetic and bioavailability studies completed and ongoing.

VIII. Clinical Studies:

A. Proposed Basic Studies:

The basic clinical program included two comparative studies in patients undergoing multiple follicular development for IVF-ET (GF 5503 and two comparative studies in WHO group II anovulatory infertile patients undergoing stimulation of follicular development and ovulation induction . As of the cut-off date for this NDA, Studies as well as six additional studies, were ongoing. Studies are being conducted in the U.S. under IND Data reporting GONAL-F efficacy are provided in this NDA for one adequate and well-controlled Phase III multicenter study (GF 5503) comparing the safety and efficacy of GONAL-F with Metrodin® (urofollitropin for injection) for stimulation of multiple follicular development in female patients undergoing in vitro fertilization and embryo transfer (IVF-ET). This is an open, randomized, parallel group, multicenter study which was conducted in Europe in accordance with EEC guidelines for Good Clinical Practice and is the only completed study which can be evaluated.

B. Design of Study GF 5503:

Ovarian follicular recruitment and development is the direct pharmacological consequence of FSH administration. In a previous study with Metrodin® in IVF-ET the mean number of preovulatory follicles (defined as the total number of follicles ≥ 14mm in diameter on the day of hCG) was 7.6 with a standard deviation of 3.7. It was considered that a difference between

Gonal-F™ and Metrodin® of 2.0 or less in the mean number of preovulatory follicles would not be clinically important.

Therefore, the number of patients was chosen so that a difference of 2.0 or more preovulatory follicles would have a high probability of being detected. A total of 106 evaluable patients were required to give 80% power to detect this difference at a two-sided significance of 0.05. Therefore, it was concluded that at least 120 patients would be randomized to allow for non-evaluable patients.

Within three months of study entry, patients were assessed for eligibility. Once a patient was deemed eligible, she was allocated to a treatment group (Gonal-F™ or Metrodin®) according to a computer generated randomization sequence using a sealed envelope procedure. At mid-luteal phase of a spontaneous cycle, treatment with GnRH agonist was started (buserelin 200 mcg/day SC) for inducing pituitary gonadotrope cell desensitization to control endogenous secretion of LH during the superovulation treatment. On days 3 to 5 of subsequent menstruation, Gonal-F™ or Metrodin® treatment was started at an initial dose of 225 IU FSH/day. Dose adaptation was allowed if necessary after five days of stimulation based upon the ovarian response to FSH therapy as assessed by serum E_2 levels and follicle growth by ultrasound. When the follicular response was judged to be adequate, final follicular maturation was induced by administration of hCG. Oocytes were retrieved, fertilized in vitro and some embryos replaced. Afterwards, the patient was followed up and the outcome (pregnancy or menstruation) was recorded.

For efficacy assessment, the protocol defined the primary end point as the number of follicles ≥ 14 mm on the day of hCG injection. The main secondary endpoints were the total number of follicles > 10 mm, the dynamics of follicular growth, serum E_2, and inhibin levels on the day of hCG, follicular phase serum FSH and E_2 levels, the numbers of oocytes retrieved, the number and percentage of oocytes fertilized, the number of embryos cleaved, the pregnancy rates and pregnancy outcomes. The total FSH dose and length of treatment to reach full follicular development were also compared between treatment groups.

Safety assessment was performed by recording adverse events, performing a general physical examination including vital signs before and after treatment completion, assessing local tolerance to study drug administration, performing hematology, biochemistry and urinalysis tests before and after treatment, and assaying anti-

FSH antibody in serum samples drawn before and after treatment completion.

C. Study population in Study GF 5503:

One hundred and twenty four patients were randomized after eligibility assessment and treated in this study. Sixty patients were treated with Gonal-F™, 63 with Metrodin® and one patient received both Gonal-F™ and Metrodin® by mistake (patient

Two efficacy analyses were performed:

(1) The "All Patients Analysis" considered all patients according to the treatment they actually received except the one patient who received both treatments. This analysis included 60 patients treated with Gonal-F™ and 63 patients treated with Metrodin®. This All Patients Analysis is considered as the primary efficacy analysis.

(2) The "Evaluable Patients Analysis" considered patients from all centers but after exclusion of those who showed major protocol violations. Fifty-eight patients were evaluable for this analysis in the Gonal-F™ group and 57 patients in the Metrodin® group.

Since three centers entered less than the 10 patients aimed for in the protocol, the All Patients Analysis and the Evaluable Patients Analysis were also performed including only patients from centers with at least 10 patients.

No significant differences were observed between the All Patients Analysis and the Evaluable Patients Analysis. For sake of simplicity, the All Patients Analysis only will be summarized and discussed hereunder.

D. Patients characteristics in Study GF 5503:

Demographic and baseline characteristics of patients in the Gonal-F™ and Metrodin® groups were similar. Moreover, treatment groups were also found to be similar in terms of number of previous ART treatments and ovarian response during previous ART treatment. The patient's partner's semen was used for in vitro fertilization in 109 cases. Donor sperm was used in 15 cases, 8 and 7 in the Gonal-F™ and Metrodin® groups respectively.

The male partner semen characteristics determined at pre-study level and on the day of OPU were found to be comparable in the two treatment groups.

compared. Thirteen babies were born in the Gonal-F™ group and 13 in the Metrodin® group. No congenital malformations were recorded in the Gonal-F™ group babies but one multiple congenital malformation was reported in the Metrodin® group babies (a male baby with a Prune Belly syndrome).

IVF results are listed below for Study GF 5503:

Variables		Gonal-F™	Metrodin®	Tests and p value for treatment effect
No of oocytes recovered	n mean SD	55 9.3 5.0	59 10.7 5.3	ANOVA : 0.35
No. of fertilized oocytes	n mean SD	55 5.6 3.8	59 6.5 4.3	ANOVA : 0.43
No. of patients with ≥ 1 fertilized oocyte	yes no	53 (96%) 2 (4%)	52 (88%) 7 (12%)	CMH : 0.068
No. of cleaved embryos	n mean SD	53 5.0 3.0	52 6.1 3.4	ANOVA: 0.12
No. of patients for each no. of transferred embryos	1 2 3 4 5	0 12 (24%) 34 (68%) 4 (8%) 0	4 (8%) 6 (11.5%) 35 (67%) 6 (11.5%) 1 (2%)	CMH : 0.77

Implantation and Pregnancies (Fresh Embryo Transfer Only) are listed below for Study GF 5503:

Variable		Gonal-F™	Metrodin®	Tests and p values for treatment effect
All pregnancies	yes no	13 37	11 41	CMH : 0.30
Implantation rate (% of replaced embryos implanted)	n mean SD	50 13% 23%	52 13% 28%	N.A.
Biochemical pregnancies	yes no	1 49	1 51	N.A.
Clinical pregnancies	yes no	12 38	10 42	CMH : 0.22
Multiple pregnancies	single twin triplet	6 6 0	6 2 2	Fisher : 0.15
Clinical abortions	yes no	2 48	1 51	N.A.
Extra-uterine pregnancies	yes no	1 49	0 52	N.A.
Dead in utero ≥ 26 weeks of gestation	yes no	0 50	1 51	N.A.
No. of patients who delivered at least one live baby	yes no	9 41	8 44	CMH: 0.37
No of babies born	yes no	13 0	13 0	N.A.

F. Safety Analysis of Study GF 5503:

The safety profile of Gonal-F™ was compared with the safety profile of Metrodin® during this clinical trial. The mean duration of exposure (9.9 ± 2.3 and 9.4 ± 1.8 days) (ANOVA, p= 0.22) and the mean total dose of FSH administered (2270 ± 714 IU and 2095 ± 591 IU) (ANOVA, p= 0.16) were similar for Gonal-F™ and Metrodin® respectively.

Treatment was not stopped in any patients for an adverse event in either the Gonal-F™ or Metrodin® treatment groups.

Four serious adverse events were reported during this clinical study.

One serious adverse event was reported among the patients treated with Gonal-F™. It was an ectopic tubal twin pregnancy which required surgery.

Three serious adverse events were reported in patients treated with

Metrodin®. One pregnant patient died at 7 months of pregnancy in the context of a bronchial asthma attack. One patient underwent abdominal surgery for abdominal abscess associated with pelvic inflammatory disease six weeks after completion of the study cycle. One patient delivered twins at 37 weeks of gestation. One male baby showed multiple congenital malformations described as the Prune Belly syndrome (urethral hypoplasia and abdominal wall deficiency).

None of these four serious adverse events were considered to be directly related to the study either by the investigator or by the study sponsor. All of these events occurred at least 38 days after FSH treatment.

Local tolerance to drug administration was assessed.

The assessment of local tolerance was based on collection of information by asking each patient to record daily on a diary card any of the following five symptoms perceived after each injection: itching, swelling, redness, bruising and pain. The severity of the symptoms were graduated as follows: none, mild, moderate, or severe. Gonal-F™ was administered subcutaneously in the abdominal wall and Metrodin® intramuscularly in the buttock. Both drugs were well tolerated since 500/575 (87%) subcutaneous injections of Gonal-F™ and 540/563 (96%) intramuscular injections of Metrodin® led to either no or only mild reactions. In addition, about 80% of patients in both treatment groups experienced either no or only mild reactions to injection throughout the treatment. The local reaction profile to Gonal-F™ was not significantly different from the local reaction profile to Metrodin® for all symptoms considered except for redness which was more marked in Gonal-F™ patients than in Metrodin® patients.

Assessment of hematology and biochemistry parameters before and after therapy did not show any significant differences between treatment groups from baseline. Hematology tests showed a moderate reduction of red blood cell count, hemoglobin and hematocrit after treatment. This may be attributable to repeated blood sampling during treatment for ovarian response monitoring and possibly also to hemodilution resulting from elevated E_2 levels. Total white blood cell counts and neutrophil percentages increased after treatment. This was similar in both groups and reflects the well known impact of repeated parenteral injections as well as the mild inflammatory syndrome usually observed in

patients with hyperstimulated ovaries. Biochemistry parameter changes from baseline were not clinically relevant. Finally, these standard laboratory tests did not show signs of hematological toxicity, significant immune reaction or systemic toxicity.

To assess the possible immunogenicity of the FSH preparations, sera were collected for centralized testing before and after treatment. Sera from all patients were tested using an immunoassay designed and validated to detect the presence of antibodies to FSH. All tested sera were found to be negative both in patients treated with Gonal-F™ and those treated with Metrodin®. In addition, no symptoms suggesting immune reactions such as fever, arthralgia or myalgia were reported and the incidence of failure to respond to therapy was low and similar in the two treatment groups. Together, these observations support Gonal-F™'s lack of immunogencity.

Among the non-serious adverse events, the most frequent was ovarian cysts (3 cases [5% of patients] in the Gonal-F™ arm and 8 cases [13% of patients] in the Metrodin® arm). Nausea, headaches and dizziness was often part of a more general clinical picture, and possibly related to elevated E_2 levels resulting from the ovarian stimulation. This is confirmed by the fact that these symptoms were equally observed in both treatment arms. The numbers of adverse events in both groups were too small to allow a relevant statistical comparison of occurrence rates. None of the serious adverse events in either group was considered drug related.

G. Overall Safety Analysis:

Gonal-F™ has been used in 12 clinical studies for different indications. Data on 232 patients have been collected, totalling 115.1 person-months of Gonal-F™ treatment. A first group of trials were clinical pharmacology studies (GF 5007, 5117, A second group of trials studied Gonal-F™ in the stimulation of ovarian follicular development either for IVF-ET (GF 5503. , or for induction of ovulation in WHO group II anovulation . In these clinical studies, one hundred and eighty different women were treated in a total number of 193 cycles. Another indication for Gonal-F™ was in male infertility (10 patients)

Safety information, including clinical laboratory data and non-serious adverse events, is available from three completed studies i.e. two phase I studies (GF 5007 & 5117), and the completed adequate and well controlled, European study (GF 5503).

Information on serious adverse events (including adverse dropouts) from the three completed and nine ongoing clinical studies were collected and have been included in the overall safety analysis.

Duration of treatment with Gonal-F™ was determined by the indication. The numbers of patients and the extent of exposure is represented below:

INDICATION	No of Patients (%)	Exposure in person-months (%)	Mean duration of exposure per patient	Mean no of treatment days per cycle
Clinical Pharmacology	42 (18.1)	6.9 (6.0)	5.0 days	-
IVF-ET	124 (53.4)	36.1 (36.4)	8.9 days	8.9 days
WHO group II anovulation	56 (24.1)	46.2 (40.1)	25.2 days	19.1 days
Male infertility	10 (4.3)	25.9 (22.5)	79.0 days	-
TOTAL	232 (100)	115.1 (100)	-	-

The overall safety profile of Gonal-F™ is similar to that determined in Study GF 5503.

IX. Literature Reviewed: All key published articles.

X. Labeling Evaluation: The labeling is essentially a replicate of the Metrodin® package insert, but not entirely.

XI. Consultation: None.

XII. Conclusions:
The production of FSH by recombinant technology coupled with a highly effective purification process, is of benefit to patients since it will provide a pure, consistent, well characterized pharmaceutical preparation of FSH.

For clinicians and their patients, the advantages of Gonal-F™ over existing FSH preparations can be summarized as follows:

- Improved batch-to-batch consistency and quality in manufacture which may enhance somewhat the consistency of individual patient's response to therapy.

- High purity readily allowing subcutaneous self-administration at home. This may contribute to improved patient treatment convenience. (but may also increase injection site reactions).

- Absence of non-FSH urinary proteins. Postmarketing surveillance will allow an assessment of the putative relationship between the rare but sometimes severe allergic reactions that have been reported with previous human gonadotropin preparations containing large amounts of co-purified human non-FSH and non-LH urinary proteins.

- Complete absence of LH activity allowing strict monotherapy until hCG is administered.

An adequate and well-controlled, multicenter, open, randomized, comparative Phase III clinical study (GF 5503) was conducted in Europe according to EEC guidelines on Good Clinical Practice including written Informed Consent. This study demonstrated that Gonal-F™ is as effective as u-hFSH (Metrodin®) in stimulating ovarian follicular development in women. The study was performed in patients undergoing in vitro fertilization and embryo transfer (IVF-ET) who were co-treated with a GnRH agonist for suppression of endogenous LH and FSH secretion. Sixty patients were treated with Gonal-F™ and 63 with Metrodin®. One patient received both treatments. The total dose of FSH and the duration of FSH therapy to achieve full follicular maturation were comparable for the two preparations. Overall follicular growth, oocyte recovery, embryo formation, embryo implantation and pregnancy rate were essentially similar in both treatment groups. No deaths were recorded in the 12 completed and ongoing studies, in Gonal-F™ treated patients.

No adverse drop-out was recorded in the three completed studies. One adverse drop-out was reported in an ongoing study (due to an ovarian cyst in study

Out of 232 patients exposed to Gonal-F™ in the twelve studies, one serious adverse event was reported. This patient , from study GF 5503 had an ectopic tubal pregnancy which required a salpingectomy.

Several serious adverse events have been reported during post-marketing surveillance of hMG and u-hFSH. These are severe ovarian hyperstimulation syndrome (OHSS), adnexal torsion, and pulmonary and vascular complications. None of these serious adverse events had been recorded in the 232 patients exposed to Gonal-F™. Since the mechanism of action of Gonal-F™ is similar to the mechanism of action of u-hFSH, it is expected that similar serious adverse events could be observed with Gonal-F™ in the future. Post-marketing surveillance should establish the actual incidence of rare adverse events such as these when using Gonal-F™. In the meantime it should be stressed that the precautions and monitoring applicable to Metrodin® treatment should also apply to Gonal-F™.

The clinical data presented in this NDA show that both safety and efficacy results for 60 patients treated with Gonal-F™ by subcutaneous injection were comparable with those for 63 patients treated with Metrodin® by intramuscular injection.

Serono has demonstrated that Gonal-F™ is similar to native FSH and in particular to urinary hFSH preparations which have been used in clinical practice for some time.

In terms of efficacy, (i) the pharmacokinetic characteristics of Gonal-F™ have been found to be similar to those of u-hFSH, and (ii) Gonal-F™ has been found to be as effective as u-hFSH in the stimulation of ovarian follicular development.

Gonal-F™ appears to be at least as safe as urinary hFSH preparations.

In clinical use, the indications for Gonal-F™, its doses and schedules of administration should be similar to those of urinary hFSH preparations. Treatment monitoring and precautions should be the same as those currently used for urinary hFSH.

XIII. Safety Update: The safety update report dated January 14, 1994 confirms the safety profile reported in the original NDA submission.

Gonal-F™ has now been used in 22 clinical studies (3 completed and 19 ongoing) in different indications. Two of the completed studies are clinical pharmacology studies. Data has now been collected on 533 patients/subjects, totaling 413 person-months of Gonal-F™ treatment:

INDICATION	NO. SUBJECTS	PERSON MONTHS
IVF-ET	202	55.2
Pharm	68	11.3
WHO II	227	227.4
Males	36	119.1
TOTAL	533	413.0

A full reanalysis of the safety data was performed for this safety report. The safety profile of Gonal-F™ did not change during this updating period. No postmarketing data is available since Gonal-F™ is not marketed anywhere in the world.

XIV. Recommendation: Approval of this application is recommended provided labeling is revised as suggested under LABELING EVALUATION.

Ridgely C. Bennett

Ridgely C. Bennett, M.D., M.P.H.

3.3.94

EXHIBIT 4

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

TRANSMITTED BY FACSIMILE

Michael I. Bernhard, Ph.D.
Senior Director, Regulatory Affairs
Ferring Pharmaceuticals
120 White Plains Road, Suite 400
Tarrytown, NY 10591

RE: Promotion of Gonal-F

Dear Dr. Bernhard:

This letter responds to your June 5, 2002, complaint to the Division of Drug Marketing, Advertising, and Communications (DDMAC), regarding claims on a current promotional brochure for Gonal-F. Thank you for the materials you have provided.

DDMAC has considered your complaint and has determined that it appears to have merit. Thus, we will take an appropriate action as deemed necessary.

We encourage you to bring to our attention other promotional materials that you believe to be false or misleading. If you have any further questions or comments, please contact me by facsimile at (301) 594-6771, or by written communication at the Food and Drug Administration, Division of Drug Marketing, Advertising, and Communications, HFD-42, Rm. 17B-20, 5600 Fishers Lane, Rockville, MD 20857. We remind you that only written communications are considered official.

Sincerely,

{See appended electronic signature page}

Sonny Saini, Pharm.D.
Regulatory Review Officer
Division of Drug Marketing,
Advertising, and Communications

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Sonny Saini
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